ORIGINAL ARTICLE

Effects of glucose and meal ingestion on incretin secretion in Japanese subjects with normal glucose tolerance

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ABSTRACT

Aims/Introduction: Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the major incretins; their secretion after various nutrient loads are well-evaluated in Caucasians. However, little is known of the relationship between incretin secretion and differing nutritional loading in Japanese subjects. In the present study, we evaluated GIP and GLP-1 secretion in Japanese subjects with normal glucose tolerance (NGT) after glucose loading (75 g glucose and 17 g glucose) and meal ingestion.

Materials and Methods: A total of 10 Japanese NGT subjects participated in 75 g oral glucose tolerance test (OGTT), 17 g OGTT and meal tolerance test (MTT). Plasma glucose (PG), serum insulin (IRI), serum C-peptide (CPR), plasma total GIP, and plasma total GLP-1 levels during OGTT and MTT were determined.

Results: Area under the curve (AUC)-GIP was increased in proportion to the amount of glucose, and was highest in MTT, showing that GIP secretion is also stimulated by nutrients other than glucose, such as lipid. In contrast, although the larger glucose load tended to induce a larger GLP-1 release, AUC-GLP-1 was not significantly different among the three loading tests (75 g OGTT, 17 g OGTT, MTT) irrespective of the kind or amount of nutrition load.

Conclusions: Our results suggest that nutritional composition might have a greater effect on GIP secretion than that on GLP-1 secretion in Japanese NGT subjects. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00143.x, 2012)

KEY WORDS: Incretin, Meal tolerance test, Oral glucose tolerance test

INTRODUCTION

Oral glucose administration leads to greater insulin release from pancreatic islets than that by intravenous glucose loading yielding equivalent glucose levels. Gut hormonal substances released in response to glucose include the incretins, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which are responsible for 50–60% of postprandial insulin secretion[1]. GIP is secreted on meal ingestion from K-cells in the proximal small intestine, whereas GLP-1 is secreted from L-cells in the distal small intestine and colon, and binds to their respective receptors on the surface of pancreatic β-cells to stimulate insulin secretion by increasing the intracellular adenosine 3′,5′-monophosphate concentration[2].

The incretin effect has been shown to be reduced in type 2 diabetic patients compared with that in normal glucose tolerance (NGT) subjects in previous studies[3,4], suggesting that a reduced incretin effect might be associated with hyperglycemia after food intake and glucose loading in type 2 diabetes. Plasma GLP-1 concentrations in type 2 diabetic patients have been reported to be reduced after meal ingestion and glucose loading[4,5]. However, in other studies, it was reported that GLP-1 concentrations did not differ in NGT and type 2 diabetic patients[6–8]. When intravenous infusion of GIP or GLP-1 was carried out in type 2 diabetic patients, GLP-1 potentiated insulin secretion from pancreatic β-cells, but GIP did not, showing that the GIP receptor (GIPR) signal is reduced in β-cells in type 2 diabetes[9]. In contrast, the GIPR signal plays an important role in maintaining blood glucose levels in the non-diabetic obese state[10,11]. Indeed, GIP concentrations are reported to be increased in obese rodent models and obese Caucasian subjects compared with those in lean rodents and lean Caucasian subjects, respectively[12–14]. In addition, we have previously shown hypersensitivity of GIPR to GIP in β-cells of high fat-induced obese mice[11]. In summary, evaluation of incretin secretion and the incretin effect in subjects with various levels of glucose tolerance is important to determine the contribution of incretin deficiency in progression from NGT to type 2 diabetes.

Type 2 diabetes is characterized by both decreased insulin secretion and reduced insulin sensitivity[15–17]. In Caucasians,

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insulin resistance is thought to play a critical role in the pathogenesis of type 2 diabetes. In contrast, insulin sensitivity in Asian subjects has been shown to be higher than that in Mexican Americans and Caucasians in previous reports[18,19], which is partly because of the fact that Asians, including Japanese, are generally less obese. Thus, insulin secretion rather than insulin sensitivity is considered to be the more important factor in progression from NGT to diabetes in Japanese subjects[20]. Indeed, we have reported that early-phase insulin secretion is considerably decreased even in Japanese NGT subjects with 1-h plasma glucose levels higher than 10 mmol/L during an oral glucose tolerance test (OGTT)[21].

A recent study showed that, in both Caucasian NGT subjects and Caucasian type 2 diabetic patients, a meal tolerance test (MTT) elicited a significantly greater response of GIP levels than that elicited by OGTT, whereas GLP-1 levels were not different between OGTT and MTT[6]. In a previous study comparing the incretin secretion measured after different amounts of glucose load in healthy Caucasian subjects and type 2 diabetic Caucasian patients, GLP-1 and GIP were dose-dependently increased[22]. Plasma GLP-1 and GIP levels after glucose load or meal ingestion have been evaluated mainly in Caucasian subjects. In Japanese subjects, there has not been thorough elucidation, and little is known about the relationship between incretin secretion, and the kind and amount of nutrition load.

In the present study, we investigated incretin levels in association with the amount of glucose load and meal ingestion by measuring plasma GLP-1 and GIP levels after administration of 17 or 75 g glucose or mixed meal in Japanese NGT subjects.

MATERIALS AND METHODS

Subjects

A total of 10 healthy Japanese volunteers (eight male and two female) were recruited into the present study. The subjects had no history of hypertension, hyperlipidemia or kidney and liver diseases, and did not take any drugs 2 weeks before the study. The study was designed in compliance with the ethics regulations of the Helsinki Declaration and Kyoto University. Informed consent was obtained from all subjects.

Study Procedure

The subjects' age, height and bodyweight were determined. Blood samples for measurement of liver and kidney function, HbA_{1c} (National Glycohemoglobin Standardization Program), triglycerides (TG), total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were drawn after an overnight fast. All subjects received 75 g OGTT, 17 g (approximately a quarter of 75 g) OGTT and a MTT. The interval between tests was 2–4 weeks. The total caloric content of the test meal was 450 kcal (carbohydrates 57.8 g, protein 17.2 g, fat 16.6 g). After the subjects fasted overnight for 10–16 h, OGTT or MTT was carried out according to the National Diabetes Data Group recommendations[23]. NGT was diagnosed according to World Health Organization (WHO) criteria[24].

Blood samples were collected at 0, 30, 60, 120 and 180 min after glucose loadings or meal ingestion and were centrifuged at 1800 *g* at 4°C for 10 min. After collecting supernatant of the samples, plasma and serum were stocked at −80°C. Blood was distributed into chilled tubes containing ethylenediaminetetraacetic acid and aprotinin (500 kIU/mL blood, Trasylol; SRL Inc., Tokyo, Japan) for analyses of GLP-1 and GIP. Plasma glucose (PG), serum insulin (IRI), serum C-peptide (CPR), plasma total GIP and plasma total GLP-1 were measured at the indicated times. The PG levels were measured by the glucose oxidase method. Serum IRI and CPR levels were measured by enzyme-linked immunosorbent assay. Total GIP and total GLP-1 levels were measured using a human GIP ELISA kit (Linco Research, St Charles, MO, USA) and human GLP-1 ELISA kit (Meso Scale Discovery, Gaithersburg, MD, USA), respectively, as previously described[25].

Calculations and Statistical Analysis

The area under the curve of PG (AUC-PG), IRI (AUC-IRI), CPR (AUC-CPR), total GIP (AUC-GIP) and total GLP-1 (AUC-GLP-1) were calculated by the trapezoidal rule. Statistical analyses were carried out using ANOVA and unpaired Student's *t*-test. *P*-values <0.05 were considered statistically significant. Data are presented as mean ± standard error (SE).

RESULTS

The profiles of the subjects are shown in Table 1. Mean age was 32.2 ± 2.0 years and mean body mass index was 22.4 ± 0.8 kg/m^2. Insulinogenic index, homeostasis model assessment (HOMA)-β and HOMA-insulin resistance were 0.59 ± 0.10, 76.50 ± 12.60, 1.10 ± 0.19, respectively. No subjects had liver or kidney dysfunction. HbA_{1c}, PG, TG, total cholesterol and HDL-cholesterol levels were within normal limits in the fasting state.

The profiles of PG, IRI and CPR in 75 g OGTT, 17 g OGTT and MTT are shown in Figure 1. Judging by the results of 75 g OGTT, all the subjects were diagnosed with NGT according to WHO criteria with fasting plasma glucose and 2 h glucose levels below 6.1 and 7.8 mmol/L, respectively. Fasting concentrations of PG, IRI and CPR were not different among the two OGTT and

Table 1 | Clinical characteristics of the subjects

n (Male/female)	10 (8/2)
Age (years)	32.2 ± 2.0
BMI (kg/m^2)	22.4 ± 0.8
Fasting plasma glucose (mmol/L)	4.9 ± 0.2
HbA_{1c} (%)	5.3 ± 0.1
Triglycerides (mg/dL)	79.4 ± 10.5
Total cholesterol (mg/dL)	169.2 ± 6.1
HDL-cholesterol (mg/dL)	61.5 ± 5.3
LDL-cholesterol (mg/dL)	93.0 ± 9.2

Data represent the mean ± SD. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

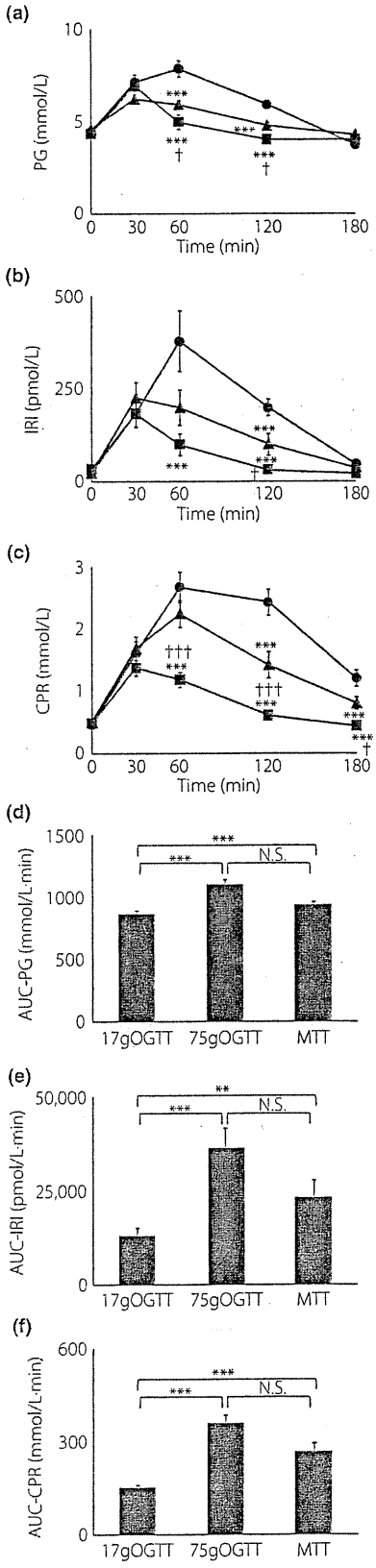

Figure 1 | Concentrations of (a) plasma glucose (PG), (b) serum insulin (IRI) and (c) serum C-peptide (CPR) during the 75 g oral glucose tolerance test (OGTT; closed circle), 17 g OGTT (closed square) and meal tolerance test (MTT; closed triangle) in 10 Japanese subjects. Asterisks indicate significant differences vs 75 g OGTT at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001); daggers indicate significant differences vs MTT at individual time-points (†P < 0.05, ††P < 0.01, †††P < 0.001). (d) Area under the curve (AUC)-PG, (e) AUC-IRI, (f) AUC-CPR were calculated by the trapezoidal rule. Asterisks indicate significant differences at individual time-points (*P < 0.05, **P < 0.01, ***P < 0.001). Statistical analyses were carried out using ANOVA and unpaired Student's t-test. P-values <0.05 were considered statistically significant. Data are presented as mean ± standard error. N.S., not significant.

MTT. In OGTT studies, AUC-PG, AUC-IRI and AUC-CPR measured by the 75 g OGTT were significantly larger than those measured by the 17 g OGTT (Figure 1d–f). At 30 min after glucose ingestion, the levels of PG, IRI and CPR in the 75 g OGTT and those in the 17 g OGTT were not significantly different. Between MTT and the two OGTT, AUC-PG, AUC-IRI and AUC-CPR in MTT were significantly higher than those in the 17 g OGTT. AUC-PG, AUC-IRI and AUC-CPR in the 75 g OGTT and in MTT were not significantly different.

In the 17 g OGTT, the total GLP-1 level peaked at 30 min and rapidly decreased to the baseline at 60 min after the glucose load. The total GLP-1 level peaked at 30 min after the meal load and was sustained for up to 180 min. In the 75 g OGTT, the GLP-1 level peaked at 60 min and gradually decreased with time, but the level was still higher than baseline even at 180 min. The level of total GLP-1 at 60 min after the 75 g glucose load was significantly higher than that after the 17 g glucose load (Figure 2a). Although a larger glucose load tended to induce a larger GLP-1 release, total AUC-GLP-1 measured by the 75 g OGTT, 17 g OGTT and MTT were not significantly different (Figure 2b).

The baseline levels of GIP were approximately 10 pmol/L. The GIP level rapidly increased, peaked at 30 min after the meal load and gradually decreased with time, but the level was still higher than baseline even at 180 min. In the 75 g OGTT, the GIP level significantly increased at 30 min after the glucose load, peaked at 120 min and were maintained up to 180 min. In the 17 g OGTT, the total GIP level peaked at 30 min after glucose load and gradually decreased to baseline at 180 min. At 30 min after ingestion, total GIP levels in the 75 g OGTT and those in the 17 g OGTT were not significantly different (Figure 3a).

AUC-GIP was significantly higher in the 75 g OGTT than that in the 17 g OGTT. Unlike GLP-1, the peak levels of GIP and the AUC-GIP measured in the MTT were significantly higher than those measured in the 75 g OGTT and 17 g OGTT (Figure 3b).

DISCUSSION

In the present study, incretin levels were estimated after glucose loading or meal ingestion in Japanese NGT subjects.

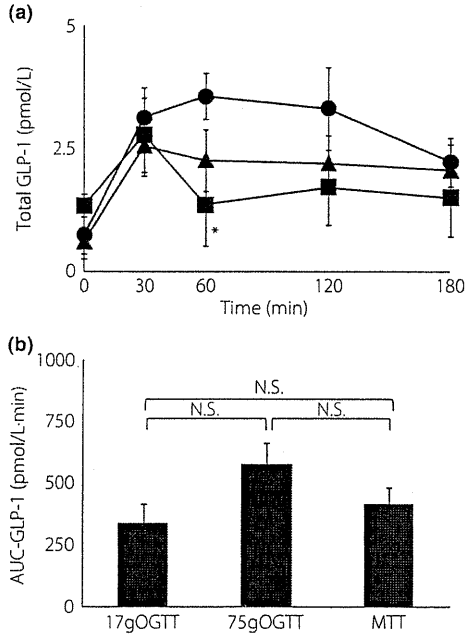

Figure 2 | (a) Concentrations of total glucagon-like peptide-1 (GLP-1) during the 75 g oral glucose tolerance test (OGTT; closed circle), 17 g OGTT(closed square) and meal tolerance test (MTT; closed triangle) in 10 Japanese subjects. Asterisks indicate significant differences vs 75 g OGTT at individual time-points (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); daggers indicate significant differences vs MTT at individual time-points (†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$). (b) Area under the curve (AUC)-GLP-1 was calculated by the trapezoidal rule. Asterisks indicate significant differences at individual time-points (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Statistical analyses were carried out using ANOVA and unpaired Student's *t*-test. *P*-values <0.05 were considered statistically significant. Data are presented as mean ± standard error. N.S., not significant.

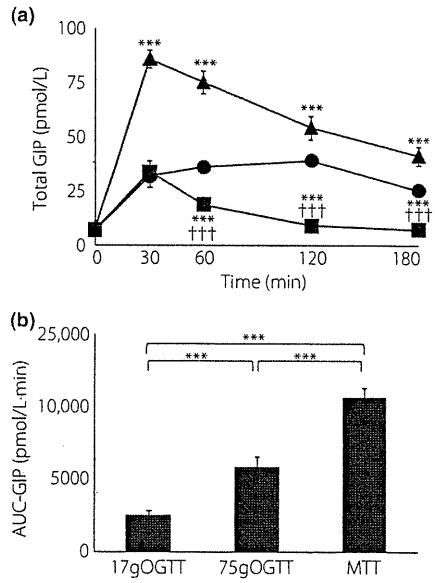

Figure 3 | (a) Concentrations of total gastric inhibitory polypeptide (GIP) during 75 g oral glucose tolerance test (OGTT; closed circle), 17 g OGTT (closed square) and meal tolerance test (MTT; closed triangle) in 10 Japanese subjects. Asterisks indicate significant differences vs 75 g OGTT at individual time-points (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); daggers indicate significant differences vs MTT at individual time-points (†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$). (b) Area under the curve (AUC)-GIP was calculated by the trapezoidal rule. Asterisks indicate significant differences at individual time-points (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Statistical analyses were carried out using ANOVA and unpaired Student's *t*-test. *P*-values <0.05 were considered statistically significant. Data are presented as mean ± standard error.

Between the OGTT studies, AUC-PG, AUC-IRI and AUC-CPR in the 75 g OGTT were larger than those in the 17 g OGTT. Regarding incretins, AUC-GIP was significantly larger in the 75 g OGTT than in the 17 g OGTT. In contrast, AUC-GLP-1 was not significantly different between the 75 g OGTT and the 17 g OGTT. Previous studies showed that a larger amount of oral glucose load elicited more GIP and GLP-1 secretion[1,22], whereas a recent study also reported that the secretory response of GIP was more sensitive than that of GLP-1 to changes in intestinal carbohydrate content[26]. The present study also showed that while GLP-1 level was not increased, GIP level was increased dose-dependently in response to glucose load, showing higher sensitivity of GIP to changes of administered nutrient dose.

Between the 75 g OGTT and MTT studies, AUC-PG, AUC-IRI and AUC-CPR were not significantly different. AUC-GIP was significantly larger in MTT than that in the 75 g OGTT. In contrast, there was no significant difference in AUC-GLP-1 among the MTT and the two OGTT. By comparing the results of the three loading tests (75 g OGTT, 17 g OGTT, MTT), we speculate that AUC-GIP is more susceptible to the contents of each loading test than AUC-GLP-1 is. Vollmer *et al.*[6] reported that GIP responses were significantly higher in MTT than in OGTT, whereas GLP-1 levels were similar in both tests in Caucasian NGT, IGT and type 2 diabetic subjects. Because the mixed meal contains not only carbohydrates but also fat, which has been reported to stimulate GIP secretion[27–29], it is likely that the increased GIP concentrations after MTT were largely as a result of the fat content, which might have had no additional impact on GLP-1 secretion.

There are two previous reports that evaluate the incretin levels in both OGTT and MTT in Japanese NGT subjects[8,30]. However, they compared the incretin levels in 75 g glucose or meal load between NGT and type 2 diabetic subjects, but did not compare the incretin levels between 75 g glucose and meal load directly. The present study directly compared the incretin levels in the two OGTT and MTT. Our data clearly show that GIP responses were significantly higher in MTT than those in the two OGTT, whereas GLP-1 levels were not different between the two OGTT and MTT in Japanese NGT subjects.

According to the study by Yabe *et al.*[8], AUC-GIP is similar between the OGTT and MTT group in Japanese control subjects. It should be noted that the difference between GIP secretion after meal load and that after glucose load was far greater in the present study than that in the study by Yabe *et al.* The total caloric content of the test meal used in their study was 480 kcal (carbohydrates 58.4%, protein 20.8%, fat 20.8%) and that in the present study was 450 kcal (carbohydrates 51.4%, protein 15.3%, fat 33.3%). Therefore, it is possible that the higher amount of contained fat in the test meal used in the present study led to the greater response of GIP secretion in the MTT.

Fasting and peak total GLP-1 concentrations in the present study were approximately 1 pmol/L and 3.5 pmol/L, respectively, and seemed to be lower than those in some published results[8,31]. However, in other reports, total GLP-1 levels after glucose and meal load were not very different from those in the present study. Rijkelijkhuizen *et al.*[32] measured the total GLP-1 concentration with radioimmunoassay, and in their results, the fasting and peak total GLP-1 concentrations in the MTT were approximately 1 pmol/L and 4.5 pmol/L, respectively. In addition, Villareal *et al.*[33] evaluated total GLP-1 concentrations by the same method that we used in the present study, and reported that the fasting and peak total GLP-1 concentrations in OGTT were approximately 1.5 and 6 pmol/L, respectively. Judging by the data in these reports, it is not necessarily the case that total GLP-1 concentrations were extremely low in the present study.

There are some reports showing that GLP-1 secretion is dependent on meal size, especially on carbohydrate and glucose loads. Schirra *et al.*[34] reported that GLP-1 plasma levels rose from basal levels to fourfold after 50 g glucose ingestion and to eightfold after 100 g glucose ingestion. Rijkelijkhuizen *et al.*[32] showed that GLP-1 secretion is increased by the amount of carbohydrate (75 and 109 g) and not by the quantity of the meal. In the present study, however, AUC-GLP-1 was not significantly different among the three loading tests (75 g OGTT, 17 g OGTT, MTT) irrespective of kinds or amounts of nutrition load, although larger glucose load tended to induce a larger GLP-1 release. The most notable difference between the previous studies and the present study was the amount of glucose load. We compared GLP-1 secretion after administration of 17 g glucose, 75 g glucose and 57.8 g of carbohydrate contained in the meal that we used. The amount of glucose and carbohydrate load in the present study were relatively lower than those in the previous studies. It is possible that evaluation of GLP-1 secretion after larger glucose loads could be more appropriate to show the glucose dependency of GLP-1 secretion.

It is also noteworthy that the levels of PG, IRI, CPR, GIP and GLP-1 at 30 min after the 75 g OGTT and 17 g OGTT were similar to each other. In addition, the levels of IRI, CPR and GLP-1 at 30 min after MTT and the two OGTT were not significantly different. By contrast, the GIP level at 30 min after MTT was much higher than those after the 17 g OGTT and 75 g OGTT. Given the similar plasma glucose levels at 30 min after the 17 g OGTT and 75 g OGTT, it is likely that under physiological conditions, the rate at which ingested glucose emptied into the duodenum is regulated finely enough to prevent an abrupt increase in plasma glucose levels irrespective of the amount of ingested glucose. Previous studies have shown that GLP-1 secretion after a test meal or oral glucose load is associated with the rate of gastric emptying, whereas GIP secretion seems to be dependent on nutrient absorption rather than on rate of gastric emptying[34]. Accordingly, a finely regulated rate of gastric emptying might account for the similar levels of GLP-1 at 30 min after MTT, 17 g OGTT and 75 g OGTT. In contrast, the level of total GIP at 30 min after the MTT was much higher than those after the two OGTT, probably because of the presence of fat in the duodenal lumen, as fat is a forcible stimulant of GIP, as discussed earlier.

The present results clearly show that the secretion of GIP and GLP-1 are regulated by different nutrient factors. On the basis of our data, it is also suggested that nutritional composition might have a greater effect on GIP secretion than on GLP-1 secretion in Japanese NGT subjects.

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RESEARCH ARTICLE

Correlations between genetic variance and adiposity measures, and gene × gene interactions for obesity in postmenopausal Vietnamese women

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Abstract

Although environmental factors are important, there is considerable evidence that genes also have a significant role in the pathogenesis of obesity. We conducted a population-based study to investigate the relationship between candidate genes for obesity (*UCP1, UCP2, ADRA2B, ADRB3, LEPR, VDR* and *ESR1*) and adiposity measures (body mass index, body fat percentage, weight, waist circumference and waist–hip ratio) in terms of individual gene and gene × gene interaction in models unadjusted and adjusted for covariates (age, years since menopause, educational level and total energy intake). Postmenopausal women with TC genotype of *ESR1* gene had higher body fat percentage than those with TT genotype in the models unadjusted and adjusted for the covariates ($P = 0.006$ in adjusted model). In multiple logistic regression analysis, *Bsm*I and *Apa*I SNPs of *VDR* genes were significantly associated with overweight and obesity. The *UCP2–VDR Apa*I interaction to susceptibility of overweight and obesity was first observed from logistic regression analysis, and then confirmed in the multifactor dimensionality reduction method unadjusted and adjusted for the covariates. This interaction had 69.09% prediction accuracy for overweight and obesity ($P = 0.001$, sign test). In conclusion, the study suggests the significant association of *ESR1* and *VDR* genes with adiposity measures and the *UCP2–VDR Apa*I interaction to susceptibility to being overweight and obesity in postmenopausal Vietnamese women.

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Introduction

Obesity, which results from an imbalance between energy intake and expenditure, is an important cause of morbidity and mortality in developed countries, and is also becoming increasingly prevalent in the developing world. In Hanoi and Ho Chi Minh City (Vietnam), the prevalence of overweight and obesity was about 30% in both sexes, and generally increased with age (Walls *et al.* 2009). Obesity and being overweight are also major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases, osteoarthritis, cancer and mental health problems, and these chronic health conditions become more prevalent in postmenopausal women. Thus, obese postmenopausal women stand at a crossroads between living the remainder of their lives in essentially good health or facing the likely onset of chronic diseases that might have been prevented (Comuzzie and Allison 1998).

As a complex disorder, obesity includes both genetic and environmental factors in its pathogenesis. It is estimated that 40%–70% of the variation in body mass index (BMI) is heritable, while cultural and social factors may explain at least 30% of the variation (Hill and Peters 1998; Dennis 2007). Environmental factors, including increased food intake and inactive lifestyle, play important role in the increase of body weight and obesity (Swinburn *et al.* 2009). Genetic factors affecting obesity are categorized by different progresses such as: appetite stimulating group (e.g. neuropeptide Y, leptin receptor, P proopiomelanocortin); energy expenditure group (e.g. uncoupling proteins); metabolism regulating group (e.g.

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beta-2 adrenergic receptor, beta-3 adrenergic receptor); and adipogenesis (e.g., peroxisome proliferator-activated receptor, vitamin D receptor, retinoid X receptor) (Bell *et al.* 2005). However, association studies have given inconsistent results due to population specificity and possible genetic effects masked by different gene × gene and gene × environment interactions (Cooper 2003). Thus, studies in different populations are needed to confirm these relations. In addition, to the best of our knowledge, there is still a dearth of data on the genetic factors of obesity in Vietnam. Therefore, we performed a population-based study to investigate the potential association of common candidate genes with adiposity measures in postmenopausal Vietnamese women.

This is an initial study from Vietnam investigating: (i) the genotype distribution of the candidate genes including uncoupling protein 1 (*UCP1*), uncoupling protein 2 (*UCP2*), alpha-2B adrenergic receptor (*ADRA2B*), beta-3 adrenergic receptor (*ADRB3*), leptin receptor (*LEPR*), vitamin D receptor (*VDR*), and oestrogen receptor 1 (*ESR1*) in the postmenopausal women; (ii) relationship between the single nucleotide polymorphisms (SNPs) of the selected genes and adiposity measures; and (iii) possible gene × gene interaction for being overweight and obesity.

Materials and methods

Subjects

One hundred and forty healthy postmenopausal women were randomly selected from the rural population of Hai Duong province, Vietnam. Of the 140 subjects in the study, 137 (98%) were farmers and manual workers, and the others were office clerks. All subjects were healthy women who did not smoke or drink alcohol. The mean (± SD) of age, age at menarche and age at menopause of the study group were 55.6 ± 3.8, 16.7 ± 2.1 and 47.7 ± 3.4 years, respectively. The Ethics Committee of the National Institute of Nutrition, Vietnam, and the Ethnical Committee of Tokushima University, Japan, approved the study. All participants provided written informed consent before entering the study.

Measurements

All participants completed a structured questionnaire. Data were collected on current age, age at menarche, age at menopause, ethnicity, educational level, occupation, medical and reproductive history, dietary, smoking and drinking history. Lifelong occupation was defined as the occupation that the subject engaged most frequently in their life. Educational level was categorized in three groups, by number of years of schooling: low level (≤ 5 years), medium level (6–8 years), and high level (≥ 9 years). Dietary intake was measured by previous 24 h dietary recall method on three consecutive weekdays (Witschi 1998).

Anthropometric measurements including weight, height, waist and hip circumference, body fat percentage were collected. Body weight and height were measured in light clothing and without shoes to the nearest 0.1 kg and 0.1 cm respectively. Body mass index (BMI) was calculated as weight per square of height (kg/m^2). Body fat percentage was measured by bioelectrical impedance method by using OMRON scale (HBF-351, Kyoto, Japan). Overweight and obesity were classified by the BMI-value recommendations of the World Health Organization (WHO Expert Consultation 2004) and the International Obesity Task Force for Asian and Pacific Island populations, which corresponds to the BMI cutoffs of 23 and 25 kg/m^2 (International Obesity Task Force 2002).

Genotyping

Peripheral blood samples were obtained from each woman and genomic DNA was extracted from peripheral blood leukocytes, using QIA amp DNA blood kit (Qiagen GmbH, Hilden, Germany). PCR protocols and primers used for genotyping *UCP1* (rs1800592), *UCP2* (rs659366), *ADRA2B* (*12Glu9*), *ADRB3* (rs4994), *LEPR* (rs1137101), *ESR1* (*Pvu*II and *Xba*I), and the common single nucleotide polymorphisms (SNPs) of *VDR* gene (*Fok*I, *Bsm*I, *Apa*I and *Taq*I) were as described previously (Kadowaki *et al.* 1995; Sivenius *et al.* 2001; Nagai *et al.* 2003; Sesti *et al.* 2003; Binh *et al.* 2006; Mitra *et al.* 2006) with some modification (table 1).

Statistical analysis

We coded genotypes as 0, 1, and 2, depending on the number of copies of the risk alleles. Genotype frequencies were compared and tested for Hardy–Weinberg Equilibrium (HWE) by Pearson's χ^2 test or Fisher's exact test, as appropriate. A pairwise |D′| value (the absolute value for the disequilibrium parameter) that ranges from 0 (complete linkage equilibrium status) to 1.0 (complete LD status) among SNPs was measured using the software program SNPstats (Sole *et al.* 2006). Haplotype frequencies for multiple loci were estimated by the expectation-maximization method, by use of the same software program SNPstats.

Quantitative variables were checked for normal distribution and compared using one-way ANOVA or independent-sample *t*-test. We used a general linear model (GLM) for one variate to evaluate the relationships between candidate genes and adiposity measures. The raw variables were adjusted by regression for covariates of age, years since menopause, educational level and total energy intake.

Gene × gene interactions were examined using both the logistic regression model and the multifactor dimensionality reduction analysis, with and without controlling for the covariates. In the logistic regression, interactions were

Table 1. PCR protocols and primers for typing the candidate genes.

Gene	dbSNP[a]	Primers	Tm	Restriction enzyme	Allele size (bp)
UCP1	rs1800592	5′-cttgggtagtgacaaagtat-3′	52°C	*Bcl*I	A: 470 bp
	A/G	5′-ccaaagggtcagatttctac-3′			G: 310 bp+160 bp
UCP2	rs659366	5′-cacgctgcttctgccaggac-3′	68°C	*Mlu*I	A: 360 bp
	G/A	5′-aggcgtcaggagatggaccg-3′			G: 290 bp+70 bp
ADRA2B	12Glu9	5′agggtgtttgtggggcatctcc-3′	63°C	–	Glu^{12}:112 bp
		5′-caagctgaggccggagacactg-3′			Glu^{9}:103 bp
ADRB3	rs4994	5′-cgcccaataccgccaacac-3′	61°C	*Bst*NI	C:161 bp;
	T/C	5′-ccaccaggagtcccatcacc-3′			T: 99 bp+62 bp
LEPR	rs1137101	5′-acccttaagctgggtgtcccaaatag-3′	57°C	*Msp*I	A: 421 bp
	A/G	5′-agctagcaaatatttttgtaagcaatt-3′			G: 294 bp+127 bp
ESR1	rs2234693	5′-gatatccagggttatgtggca-3′	60°C	*Pvu*II	C(P): 346 bp
	T/C	5′-aggtgttgcctattatattaaccttga-3′			T(p): 241 bp+105 bp
	rs9340799	5′-gatatccagggttatgtggca-3′	60°C	*Xba*I	G(X): 346 bp
	A/G	5′-aggtgttgcctattatattaaccttga-3′			A(x):196 bp+150 bp
VDR	rs2228570	5′-agctggccctggcactgactctgctct-3′	60°C	*Fok*I	C(F): 265 bp
	C/T	5′-atggaaacaccttgcttcttctccct-3′			T(f): 196 bp+69 bp
	rs1544410	5′-caacaaagactacaagtaccgcgtcagtga-3′	54°C	*Bsm*I	A(B): 822 bp
	G/A	5′-aaccagcggaagaggtcaaggg-3′			G(b): 646 bp+176 bp
	rs7975232	5′-cagagcatggacagggagcaag-3′	62°C	*Apa*I	A(A): 746 bp
	C/A	5′-gcaactcctcatggctgaggtctca-3′			C(a): 532 bp+214 bp
	rs731236	5′-cagagcatggacagggagcaag-3′	62°C	*Taq*I	T(T): 746 bp
	T/C	5′-gcaactcctcatggctgaggtctca-3′			C(t): 497 bp+249 bp

[a]Accession number of each polymorphism to dbSNP at http://www.ncbi.nlm.nih.gov/. Alleles underlined were proposed to be the risk alleles for obesity identified by previous studies.

assessed using a likelihood-ratio test. Here, data are presented as odds ratios with 95 per cent confidence intervals (CI). The above statistical procedures were performed using SPSS version 16.0 (SPSS, Chicago, USA). Multifactor dimensionality reduction (MDR) analysis was used to detect gene × gene interactions to obesity and overweight. A detailed explanation on MDR has been provided elsewhere (Hahn *et al.* 2003; Moore *et al.* 2006; Lou *et al.* 2007). Briefly, MDR is a genetically model-free and nonparametric alternative to logistic regression. MDR acts by reducing a set of multilocus genotypes to one dimension with two groups: a high-risk and a low-risk set of genotypes. A particular multilocus genotype can be declared to be high-risk if the ratio of number of cases to controls exceeds the proportion of cases in the total sample. By grouping the high-risk multilocus genotypes together and the low-risk genotypes together, the model is reduced to one dimension, i.e., essentially one variable with two possible values: high or low risk 2-locus genotypes. Models are evaluated on the testing balanced accuracy statistic (TBA), the cross-validation consistency (CVC), and the statistical significance of the model. The TBA measures how often individuals are correctly classified with respect to their case/control status and the CVC evaluate the consistency with which individuals are classified. We used 10000 permutations to determine the statistical significance of the best models. These data were analysed using an extension of the MDR algorithm that includes adjustment for covariates, the generalized multifactor dimensionality reduction (GMDR, v. 0.7) software package (Lou *et al.* 2007).

Results

Genotype distribution of the candidate genes

Genotype frequency of the selected SNPs in the postmenopausal women is shown in table 2. All SNPs were in Hardy–Weinberg equilibrium ($P > 0.3$), except for *ADRB3*, which was marginally significant ($P = 0.076$). In the *UCP* gene, SNP rs1800592 (*UCP1*) and SNP rs659366 (*UCP2*) were in different LD block ($D' = 0.21$, $P = 0.001$). From four SNPs of the *VDR* gene, the polymorphisms at the 3′end of the gene (*Bsm*I, *Apa*I and *Taq*I) exhibited a strong linkage disequilibrium (D' ranging from 0.91 to 0.99, $P < 0.001$), forming three frequent haplotypes: baT (66.6%), bAT (24.5%), Bat (5.7%), which jointly represent nearly 98% of all haplotypes. SNP rs2228570 (*Fok*I) was separated from the others in *VDR* gene by over 33 kb, with D' ranging from 0.014 to 0.096. In *ESR1* gene, the *Pvu*II (rs2234693) and *Xba*I (rs9340799) polymorphisms were 45 bp apart and located approximately 400-bp upstream of exon 2, and the two polymorphisms were in the same LD block ($D' = 0.99$, $P < 0.001$).

Correlations between genetic variance and adiposity measures

Table 2 shows the comparison of adiposity measures among genotypes of each candidate genes. No significant association was observed between polymorphisms of the selected SNPs and weight, BMI, body fat percentage, waist

Table 2. Comparison of adiposity measures among genotypes of each candidate gene.

Gene	Genotype	N (%)	Adiposity measures				
			Weight (kg)	BMI (kg/cm^2)	Body fat (%)	Waist circumference (cm)	Waist–hip ratio
UCP1	AA	26 (18.6)	46.5 ± 7.7	20.9 ± 2.4	31.5 ± 8.7	72.2 ± 8.3	0.83 ± 0.07
	AG	76 (54.3)	45.4 ± 6.5	20.5 ± 2.7	29.5 ± 4.1	70.1 ± 7.3	0.82 ± 0.05
	GG	38 (27.1)	45.0 ± 6.0	20.0 ± 2.5	28.9 ± 4.1	69.9 ± 7.0	0.82 ± 0.06
UCP2	AA	25 (17.9)	45.5 ± 6.6	20.6 ± 2.6	29.8 ± 4.2	71.4 ± 7.4	0.84 ± 0.06
	AG	69 (49.3)	45.7 ± 7.1	20.6 ± 2.7	29.6 ± 4.2	70.7 ± 7.9	0.82 ± 0.06
	GG	46 (32.9)	45.2 ± 5.9	20.0 ± 2.4	29.1 ± 4.2	69.6 ± 6.7	0.81 ± 0.06
ADRA2B	Glu^{12}/Glu^{12}	50 (35.7)	46.0 ± 6.6	20.4 ± 2.5	29.8 ± 3.7	71.4 ± 7.3	0.82 ± 0.06
	Glu^{12}/Glu^{9}	65 (46.4)	44.8 ± 6.5	20.2 ± 2.5	28.8 ± 4.5	69.6 ± 7.2	0.82 ± 0.05
	Glu^{9}/Glu^{9}	25 (17.9)	46.4 ± 6.8	21.0 ± 2.7	30.8 ± 4.2	70.9 ± 8.3	0.81 ± 0.07
ADRB3	TT	102 (72.9)	45.3 ± 6.7	20.4 ± 2.6	29.5 ± 4.0	70.2 ± 7.4	0.82 ± 0.06
	TC	38 (27.1)	45.9 ± 6.4	20.4 ± 2.4	29.4 ± 4.7	71.0 ± 7.5	0.82 ± 0.06
LEPR	AA	3 (2.1)	48.2 ± 3.4	21.8 ± 1.5	33.3 ± 1.9	75.6 ± 3.0	0.84 ± 0.02
	AA	28 (20)	43.9 ± 5.4	19.9 ± 2.5	29.2 ± 3.8	69.3 ± 6.6	0.81 ± 0.06
	GG	109 (77.9)	45.8 ± 6.9	20.5 ± 2.6	29.4 ± 4.3	70.6 ± 7.7	0.82 ± 0.06
ESR1	TT (pp)	52 (37.1)	44.3 ± 5.8	19.9 ± 2.2	28.3 ± 3.9	68.8 ± 6.7	0.81 ± 0.06
PvuII	TC (Pp)	66 (47.1)	46.3 ± 6.7	20.7 ± 2.8	30.3 ± 4.2*	71.4 ± 7.9	0.82 ± 0.06
	CC (PP)	22 (15.7)	46.1 ± 7.7	20.9 ± 2.6	29.9 ± 4.2	71.6 ± 7.1	0.83 ± 0.05
ESR1	AA (xx)	90 (64.3)	45.0 ± 6.4	20.2 ± 2.5	29.1 ± 4.2	70.2 ± 7.8	0.82 ± 0.06
XbaI	AG (Xx)	45 (32.1)	45.9 ± 6.7	20.6 ± 2.6	29.9 ± 4.1	70.5 ± 6.6	0.82 ± 0.05
	GG (XX)	5 (3.6)	50.5 ± 6.8	22.5 ± 3.0	32.3 ± 5.4	75.2 ± 6.1	0.83 ± 0.03
VDR	CC (FF)	39 (27.9)	45.3 ± 7.2	20.5 ± 2.5	29.7 ± 4.0	71.5 ± 8.1	0.83 ± 0.06
FokI	CT (Ff)	72 (51.4)	46.0 ± 6.5	20.6 ± 2.6	29.5 ± 4.3	70.3 ± 7.5	0.82 ± 0.06
	TT (ff)	29 (20.7)	44.6 ± 6.1	20.0 ± 2.6	29.3 ± 4.2	69.3 ± 6.2	0.81 ± 0.04
VDR	AA (BB)	1 (0.7)	39.4	17.6	29.4	64.2	0.78
BsmI	AG (Bb)	23 (16.4)	46.5 ± 8.3	20.7 ± 2.8	29.5 ± 4.7	69.5 ± 7.3	0.81 ± 0.05
	GG (bb)	116 (82.9)	45.4 ± 6.2	20.4 ± 2.5	29.5 ± 4.1	70.7 ± 7.5	0.82 ± 0.06
VDR	AA (AA)	12 (8.6)	45.2 ± 4.6	20.6 ± 1.5	30.1 ± 2.4	70.7 ± 7.0	0.83 ± 0.07
ApaI	AC (Aa)	68 (48.6)	45.3 ± 7.2	20.1 ± 2.6	29.0 ± 4.5	69.8 ± 7.6	0.82 ± 0.06
	CC (aa)	60 (42.8)	45.8 ± 6.3	20.8 ± 2.7	29.9 ± 4.1	71.2 ± 7.4	0.82 ± 0.05
VDR	TT (TT)	124 (88.6)	45.7 ± 6.5	20.5 ± 2.6	29.6 ± 4.2	70.6 ± 7.4	0.82 ± 0.06
TaqI	TC (Tt)	16 (11.4)	44.2 ± 7.3	19.6 ± 2.5	29.0 ± 4.5	69.2 ± 8.0	0.81 ± 0.05

Data are presented as mean ± SD unless otherwise indicated. P value was derived from one-way ANOVA or independent t-test, as appropriate. *$P = 0.036$ TC versus TT, the other P values > 0.05.

circumference and waist–hip ratio, except for the *Pvu*II polymorphism of *ESR1* gene (women with TC genotype had higher body fat percentage than those with TT genotype, $P = 0.036$). Moreover, the significant association between the *ESR1 Pvu*II polymorphism and body fat percentage was consistently found after adjustment for age, years since menopause, educational level and total energy intake ($P = 0.006$, TC versus TT) (table 3). Significant associations were observed in the *Bsm*I (rs1544410) and *Taq*I polymorphisms (rs731236) of *VDR* gene for both weight and BMI, and in the *ESR1 Xba*I polymorphism for BMI (table 3) in the ANCOVA analysis controlling for covariates. Further analysis on relationship between *ESR1 Pvu*II–*Xba*I haplotype and body fat percentage indicated that carries with 1 px haplotype had consistently higher body fat percentage than those with 2 px haplotype in models unadjusted and adjusted for age, years since menopause, educational level and total energy intake ($P = 0.036$ in unadjusted model and $P = 0.014$ in adjusted model, data not shown). A similar analysis showed no association between body fat percentage and number copies of the Px or PX haplotypes.

Association between candidate genes and obesity

To identify potential risk variables for obesity and being overweight, we used binary logistic regression in the models, including the SNPs selected, age, years since menopause, educational level, and total energy intake. As presented in table 4, we found no association between obesity and the tested SNPs in the models with and without adjustment for covariates. However, the *Bsm*I and *Apa*I polymorphisms of *VDR* gene were statistically significantly associated with being overweight and obesity in both unadjusted and adjusted models, taking into account the effect of the other SNPs.

Possible gene × gene interaction in predisposition of overweight and obesity

Table 5 summarizes the potential pairwise gene × gene interactions for overweight and obesity by logistic regression analysis. The *UCP2–VDR Apa*I interplay was the most significant interaction among all possible pairwise gene ×

Table 3. Analysis of covariates of adiposity measures among candidate genes.

Gene	Genotype	Adiposity measures				
		Weight (kg)	BMI (kg/cm^2)	Body fat (%)	Waist circumference (cm)	Waist–hip ratio
UCP1	AA	47.4 ± 1.9	21.3 ± 0.7	31.9 ± 1.2	73.2 ± 2.2	0.82 ± 0.02
	AG	47.1 ± 1.6	21.2 ± 0.6	31.0 ± 1.0	72.0 ± 1.9	0.82 ± 0.02
	GG	45.7 ± 1.8	20.3 ± 0.7	30.0 ± 1.1	71.2 ± 2.1	0.82 ± 0.02
UCP2	AA	46.8 ± 2.0	21.1 ± 0.8	31.3 ± 1.3	73.0 ± 2.3	0.83 ± 0.02
	AG	46.7 ± 1.6	21.1 ± 0.6	30.9 ± 1.0	72.2 ± 1.8	0.82 ± 0.01
	GG	46.6 ± 1.7	20.7 ± 0.7	30.6 ± 1.1	71.2 ± 2.0	0.81 ± 0.02
ADRB3	TT	46.2 ± 1.5	20.9 ± 0.6	30.9 ± 1.0	71.7 ± 1.8	0.82 ± 0.01
	TC	47.2 ± 1.8	21.1 ± 0.7	31.1 ± 1.1	72.5 ± 2.0	0.82 ± 0.02
LEPR	AA+AG	46.1 ± 1.9	20.9 ± 0.7	31.0 ± 1.2	72.0 ± 2.1	0.81 ± 0.02
	GG	47.3 ± 1.5	21.1 ± 0.6	30.9 ± 0.9	72.3 ± 1.7	0.82 ± 0.01
ADRA2B	Glu^{12}/Glu^{12}	46.8 ± 1.7	20.6 ± 0.7	30.8 ± 1.1	73.0 ± 2.0	0.83 ± 0.02
	Glu^{12}/Glu^{9}	45.3 ± 1.6	20.5 ± 0.6	29.9 ± 1.1	70.7 ± 1.9	0.82 ± 0.02
	Glu^{9}/Glu^{9}	48.1 ± 1.9	21.7 ± 0.7	32.2 ± 1.2	72.7 ± 2.2	0.81 ± 0.02
ESR1	TT	46.2 ± 1.9	20.6 ± 0.7	30.1 ± 1.2	70.5 ± 2.2	0.80 ± 0.02
PvuII	TC	48.4 ± 1.8	21.7 ± 0.7	32.4 ± 1.1[f]	73.8 ± 2.0	0.82 ± 0.02
	CC	45.5 ± 1.9	20.6 ± 0.7	30.3 ± 1.2	72.1 ± 2.2	0.83 ± 0.02
ESR1	AA	44.0 ± 1.4	19.8 ± 0.5	29.3 ± 0.9	70.4 ± 1.6	0.82 ± 0.01
XbaI	AG	43.9 ± 1.6	19.8 ± 0.6	29.6 ± 1.0	69.2 ± 1.9	0.81 ± 0.02
	GG	52.2 ± 3.5	23.3 ± 1.3[c]	34.0 ± 2.2	76.8 ± 4.0	0.82 ± 0.03
VDR	CC	47.2 ± 1.8	21.3 ± 0.7	31.3 ± 1.1	73.4 ± 2.1	0.83 ± 0.02
FokI	CT	47.3 ± 1.8	21.1 ± 0.7	31.1 ± 1.2	72.6 ± 2.1	0.82 ± 0.02
	TT	45.7 ± 1.8	20.4 ± 0.7	30.4 ± 1.1	70.5 ± 2.0	0.81 ± 0.02
VDR	AA + AG	49.5 ± 2.0[a]	22.2 ± 0.8[d]	31.7 ± 1.3	71.7 ± 2.3	0.81 ± 0.02
BsmI	GG	43.9 ± 2.0	19.7 ± 0.8	30.2 ± 1.3	72.6 ± 2.3	0.83 ± 0.02
VDR	AA	46.7 ± 2.3	21.0 ± 0.9	31.2 ± 1.5	72.6 ± 2.7	0.82 ± 0.02
ApaI	AC	46.5 ± 1.5	20.6 ± 0.6	30.5 ± 1.0	71.7 ± 1.8	0.81 ± 0.01
	CC	47.0 ± 1.6	21.2 ± 0.6	31.2 ± 1.1	72.2 ± 1.9	0.82 ± 0.02
VDR	TT	50.2 ± 1.9[b]	22.6 ± 0.7[e]	31.9 ± 1.2	72.5 ± 2.2	0.81 ± 0.02
TaqI	TC	43.2 ± 2.4	19.3 ± 0.9	30.0 ± 1.5	71.8 ± 2.7	0.82 ± 0.02

Data are presented as mean ± SD. *P*-value was derived from ANCOVA test with adjustment for age, years since menopause, educational level, and total energy intake. [a]$P = 0.033$, AA + AG vs. GG; [b]$P = 0.022$, TT vs. TC; [c]$P = 0.043$, GG vs. AG and AA; [d]$P = 0.013$ AA + AG vs. GG; [e]$P = 0.006$, TT vs. TC; [f]$P = 0.047$, TC vs. TT and CC.

gene interactions in the models unadjusted and adjusted for covariates (table 5).

Table 6 presents the potential gene × gene interaction in predisposition for obesity and being overweight among 11 polymorphisms, using GMDR analysis. From all possible pairwise interactions, the most significant gene × gene interplay was the *UCP2–VDR ApaI* interaction, which had 69.09% prediction accuracy for both overweight and

Table 4. Associations between SNPs in seven candidate genes with overweight and obesity.

Gene	Obesity				Overweight and obesity			
	OR (95% CI)	*P* value[a]	OR (95% CI)	*P* value[b]	OR (95% CI)	*P* value[a]	OR (95% CI)	*P* value[b]
UCP1	0.80 (0.23-2.75)	0.722	0.77 (0.22-2.75)	0.686	0.85 (0.39-1.87)	0.688	0.86 (0.39-1.93)	0.718
UCP2	0.45 (0.12-1.67)	0.232	0.42 (0.10-1.72)	0.226	0.58 (0.28-1.20)	0.142	0.60 (0.28-1.30)	0.192
ADRA2B	0.95 (0.28-3.20)	0.936	0.96 (0.29-3.22)	0.953	1.78 (0.85-3.37)	0.126	1.80 (0.83-3.90)	0.140
ADRB3	0.35 (0.03-3.74)	0.383	0.37 (0.03-4.13)	0.417	0.69 (0.22-2.19)	0.530	0.56 (0.17-1.85)	0.339
LEPR	1.99 (0.28-14.1)	0.493	1.88 (0.25-14.1)	0.536	0.57 (0.14-2.39)	0.445	0.60 (0.14-2.67)	0.505
ESR1 PvuII	0.69 (0.18-2.73)	0.600	0.70 (0.17-2.78)	0.608	0.82 (0.36-1.88)	0.635	0.78 (0.32-1.90)	0.585
ESR1 XbaI	1.92 (0.42-8.77)	0.403	2.00 (0.41-9.85)	0.393	2.16 (0.79-5.86)	0.132	2.07 (0.71-5.99)	0.181
VDR FokI	0.91 (0.28-2.98)	0.881	0.83 (0.24-2.86)	0.763	1.55 (0.73-3.30)	0.256	1.80 (0.80-4.04)	0.153
VDR BsmI	0.15 (0.01-3.74)	0.247	0.13 (0.01-3.67)	0.233	0.15 (0.02-0.93)	**0.042**	0.13 (0.02-0.91)	**0.039**
VDR ApaI	6.01 (0.70-51.9)	0.103	6.30 (0.71-56.0)	0.099	3.03 (1.10-8.34)	**0.032**	3.00 (1.08-8.36)	**0.036**
VDR TaqI	–	0.998	–	0.998	6.44 (0.65-63.7)	0.111	6.50 (0.61-69.1)	0.121

OR, odds ratio; CI, confidence interval. ORs (95% CI) were reported with respect to the risk allele using a log additive model in logistic regression. [a]Unadjusted; [b]adjusted by age, years since menopause, educational level, and total energy intake.

Table 5. The possible pairwise gene × gene interactions for overweight and obesity by logistic regression analysis.

Interacting SNPs	Model	OR (95% CI)	*P* value for interaction	*P* value for model fit (*)
UCP2-VDR ApaI	Unadjusted	0.10 (0.02-0.42)	0.002	0.001
	Adjusted	0.09 (0.02-0.39)	0.001	0.003
ESR1 PvuII-VDR FokI	Unadjusted	3.37 (1.36-8.39)	0.009	0.006
	Adjusted	3.38 (1.32-8.63)	0.011	0.023
ESR1 XbaI-VDR ApaI	Unadjusted	0.14 (0.03-0.66)	0.013	0.004
	Adjusted	0.13 (0.03-0.65)	0.013	0.017
ESR1 XbaI-VDR FokI	Unadjusted	0.37 (0.15-0.96)	0.041	0.027
	Adjusted	0.35 (0.13-0.95)	0.039	0.052
ADRB3-VDR ApaI	Unadjusted	0.14 (0.02-0.83)	0.030	0.046
	Adjusted	0.10 (0.01-0.65)	0.016	0.068

OR, odds ratio; CI, confidence interval. Odds ratios and *P* values were derived from the models unadjusted and adjusted for age, years since menopause, educational level, and total energy intake. (*) Omnibus tests of model coefficients: Model adequately fits the data if *P* value < 0.05.

obesity ($P = 0.001$, sign test), and a maximum prediction accuracy increased up to 75.36% ($P = 0.0054$, on the basis of 10000-fold permutation testing). The CVC (10/10) indicated consistency in the cross validation measures. This interaction remained essentially unchanged in the model adjusted for the covariates (age, years since menopause, educational level and total energy intake). The other possible interactions listed in table 5 were not confirmed in MDR analysis. We could not detect significant gene × gene interactions in models for obesity with and without adjustment for the covariates.

Discussion

As obesity is polygenic disorder, we included in the study the candidate genes from different pathways composed of appetite stimulating gene (*LEPR*), energy expenditure group (*UCP1* and *UCP2*), metabolism regulating group (*ADRA2B* and *ADRB3*), and adipogenesis (*VDR* and *ESR1*). Although the selected SNPs have been widely investigated in developed countries, it is the first report of the relationship between genetic factors and obesity in Vietnam. Among the 11 SNPs studied, we observed the significant association of *ESR*1 and *VDR* with adiposity measures and the *UCP2–VDR ApaI* interaction to susceptibility of overweight and obesity.

With regard to *ESR1* gene, postmenopausal women with TC genotype had higher body fat percentage than those with TT genotype in our study. This significant association also remained essentially unchanged in the models unadjusted and adjusted for the covariates (age, years since menopause, educational level and total energy intake). Although it is not in line with previous reports that the *ESR1 Pvu*II C allele and its associated genotypes and haplotypes were inversely associated with obesity in white postmenopausal women (Goulart *et al.* 2009) and *ESR1 Pvu*II polymorphism was not associated with body fat distribution and obesity in Japanese postmenopausal women (Okura *et al.* 2003), our observation can be explained by the report suggesting that the *ESR1 Pvu*II C allele was associated with increased transcription of *ESR1* when compared with the common allele and thus presumably associated with higher relative levels of functional ESR1 protein (Herrington *et al.* 2002). Further haplotype analysis showed that carries with 1 px haplotype had higher body fat percentage than those with 2 px haplotype in models unadjusted and adjusted for the covariates. This is consistent with the findings that plasma oestradiol level decreased by 1.9 pmol/L per copy number of px haplotype in postmenopausal women (Schuit *et al.* 2005), since the effect of low oestrogen on increased obesity has been linked to *ESR1* gene (Ohlsson *et al.* 2000). Furthermore *ESR1* plays a pivotal role in the regulation of food intake and energy expenditure by oestrogens. The ventromedial nucleus (VMN) of the hypothalamus has a high density of oestrogen-binding sites (Pfaff and Keiner 1973), and neurons in this nucleus express *ESR1* at high levels (Li *et al.* 1993). The VMN lesion or ovariectomy both lead to increased food intake and body weight, suggesting that *ESR1* expressed in VMN neurons is an important player in the central control of body weight by oestrogens. In addition, after *ESR1* silencing in the VMN, the mice displayed a higher increase in food consumption as early as two weeks after surgery, especially suppression of *ESR1* levels in the VMN of adult female animals triggered the development of metabolic syndrome marked by a profound increase in body weight and excess visceral fat (Musatov *et al.* 2007).

In terms of *VDR* gene, no significant association was observed between the polymorphism of individual SNPs and BMI, which is consistent with a previous report in Caucasian postmenopausal women (Tworowska-Bardzińska *et al.* 2008). However, the significant associations with BMI were found for *Bsm*I and *Taq*I polymorphisms after adjustment for the covariates. In addition, the multiple logistic regression analysis indicated that *Bsm*I and *Apa*I polymorphisms were statistically significantly associated with being overweight and obesity in both unadjusted and adjusted model, taking into account the effect of other SNPs. This points the limitation of single SNP-based association study.

Table 6. Gene × gene interaction models for overweight and obesity.

Interacting SNPs	Testing BA	CVC	*P* value
Models for overweight and obesity[a]			
UCP2-VDR ApaI	0.6909	10/10	0.001
UCP1-ADRA2B-ESR1 PvuII	0.4564	3/10	0.623
Models for overweight and obesity[b]			
UCP2-VDR ApaI	0.6965	10/10	0.011
UCP2-VDR ApaI-ESR1 XbaI	0.4783	5/10	0.377

P values are from the sign test. BA, balanced accuracy; CVC, cross validation consistency.
[a]Unadjusted; [b]adjusted by age, years since menopause, educational level, and total energy intake.

One of the major findings of the present study is to detect the most significant *UCP2–VDR ApaI* interaction to susceptibility of overweight and obesity among all possible pairwise gene × gene interactions. This interaction was first observed in logistic regression analysis, and then confirmed in the MDR method unadjusted and adjusted for the covariates (age, years since menopause, educational level and total energy intake). This interesting observation can explain partly the possible genetic effects masked by different gene × gene interaction leading to the controversial results in association studies. Although the molecular mechanisms underlying the interaction between *UCP2* gene and *VDR* gene remain to be explored, a prior study has reported suppression of *UCP2* expression by 1,25(OH)2D3 in human adipose tissues via the nuclear *VDR* (Shi *et al.* 2002) and a recent work has indicated that 1,25(OH)2D3 suppresses *UCP* expressions through direct gene regulation and that basal *UCP* expression is upregulated in the brown fat of *VDR* (–/–) mice. Thus, *VDR* interacts with UCPs in the pathogenesis of obesity, whereas the food intake is not significantly different between *VDR*-null mice and wild-type mice (Wong *et al.* 2009).

Because obesity is known as a lifestyle-related disorder, we took into account the analysis of both genetic factors and environmental factors, including: (i) reproductive status: age and years since menopause; (ii) lifestyle factors: total energy intake, educational level and occupation. As all subjects were healthy women who did not smoke and drink alcohol, and most of them (98%) were farmers and manual workers, we could eliminate the confounding factors of these variables in all analysis models.

In the present study, we used the classification of overweight and obesity according to the recommendations of the World Health Organization (WHO Expert Consultation 2004) and the International Obesity Task Force for Asian and Pacific Island populations, which corresponds to the BMI cutoffs of 23 and 25 kg/m^2 (International Obesity Task Force 2002). These recommendations were based on the presence of excessive body fat and adverse effects on health (mortality and morbidity). Studies in Indonesia, Singapore, Japan and Hong Kong demonstrated that for the same BMI, Asians living in these countries had higher body fat percentage compared to age-matched and sex-matched Caucasians with the same BMI (Guricci *et al.* 1998; Deurenberg-Yap *et al.* 2000; Gallagher *et al.* 2000; Ko *et al.* 2001), while a recent study did not support it (Ho-Pham *et al.* 2010). Although there has been disagreement on this classification, many studies have supported the use of theses cutoff to classification of overweight and obesity for Asian populations (Ko and Tang 2007; He *et al.* 2008; Yamada *et al.* 2008). Further, researches are required to derive a more appropriate BMI threshold for defining obesity and being overweight for Asians.

The present findings must be interpreted in the context of several potential limitations. First, the most important limitation was the small sample size. Although statistically significant genetic associations were indeed identified, the sample lacked the statistical power to detect other potential associations. Next, using the bioelectrical impedance method to measure body fat percentage is not known as a 'gold standard', but rather the dual-energy X-ray absorptiometry method. However, in the context of study in developing countries at the public health level, the bioelectrical impedance method has been proposed as an alternative method for noninvasive assessment of body fat percentage, especially useful for pregnant women, due to its low cost, portable tools and feasibility for a large population study.

In conclusion, the present study showed the association of VDR and ESR1 with adiposity measures and the significant *UCP2–VDR ApaI* interaction to susceptibility of overweight and obesity. For future investigations on the association between adiposity measures and genes, use of larger sample sizes, prospective study designs, and additional markers would enhance the findings.

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Letter to Editor

IUNS Workshop on Capacity and Leadership Development in Nutritional Sciences Held in Tokyo 2010

Key Words capacity building, nutritional sciences, IUNS, Japan

Dear Editor:

In order to strength the basis for more integrated and qualified research, capacity building has been part of the prioritized agenda in the societies of nutritional sciences. The International Union of Nutritional Sciences (IUNS) and United Nations University (UNU) have conducted nutrition leadership training programs for several years. Following three IUNS Nutrition Leadership Training Programs held in Asian countries (China, Korea and Taiwan) (*1*), the IUNS Committee of the Science Council of Japan held the 4th Workshop on Capacity and Leadership Development in Nutritional Sciences in Tokyo, from 7 Sep to 9 Sep 2010. The workshop was hosted by the leading Japanese societies on nutrition: The Japanese Society of Nutrition and Dietetics, The Japanese Society of Nutrition and Food Science, and the National Institute of Health and Nutrition, Japan.

A total of, 29 persons from Japan and 22 persons from other Asian countries applied to participate in the workshop in response to the announcements on the IUNS Website, and homepages of the three hosting bodies, as well as direct notices sent to the national contact points of IUNS or other relevant organizations in Asia. Among the 51 candidates, the organizing committee selected 36 participants from Japan (21 persons), Korea (3), Malaysia (3), Thailand (3), Singapore (2), Vietnam (1), Laos (1), Cambodia (1), and India (1). As faculty members, 14 scientists belonging to the three host organizations (Table 1) gave lectures, coordinated and facilitated three topics for group works.

There were (a) "*Shokuiku*" (nutrition education) at the national level: a focus on school meal programs and their related policies, (b) nutrition and community empowerment,and (c) functional foods. Further, they arranged field trips to (1) a primary school for observing the school meal program, (2) an NGO for a community-based food security program, and (3) a company in the food industry (Table 2). The 4th Asian Network Symposium on Nutrition (Theme—Nutrition Education Program for National Health Promotion in Asian Countries: A Focus on School-based Programs) hosted by the National Institute of Health and Nutrition was incorporated into the programs of the workshop in order to share the extensive information and in-depth discussions especially about the school lunch programs in Asian countries (Table 3).

All the participants completed the programs through their own presentations and enthusiastic discussions in the groups, and further personal communications in Japanese restaurants after the group work. During the final session, representatives from the three groups presented their learning through field trips and group discussions. Participants from both Japan and other Asian countries were fully satisfied with the overall outcome of the workshop, which could be linked to the development of further personal communication and collaborations to promote research activities in the Asian countries.

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Table 1. Organizers and sponsors.

1) Principal organizing members
[Representative, IUNS Committee of the Science Council of Japan] Makoto Shimizu (Professor, Graduate School of Agricultural and Life Sciences, The University of Tokyo)
[Chair of the Organizing Committee] Shigeru Yamamoto (Professor of International Nutrition, Ochanomizu University Graduate School)
[Chair of the Executive Board] Shinkan Tokudome (Director-General, National Institute of Health and Nutrition)
[Chief of the Secretariat] Nobuo Yoshiike (Professor, Department of Nutrition, Aomori University of Health and Welfare)
2) Lectures, Faculties and Coordinators
[The Japanese Society of Nutrition and Dietetics]
Yukari Takemi (Professor, Department of Nutrition Sciences, Kagawa Nutrition University)
Nobuko Murayama (Professor, Public Nutrition & International Nutrition, Niigata University of Health and Welfare)
Midori Ishikawa (Professor, Community Nutrition & International Nutrition, Nayoro City University)
Kumi Eto (Lecturer, Laboratory of Nutrition Ecology, Kagawa Nutrition University)
[The Japanese Society of Nutrition and Food Science]
Kiyoshi Tanaka (Professor, Clinical Nutrition, Department of Food and Nutrition, Kyoto Women's University)
Hisanori Kato (Project Professor, Food for Life, Organization of Interdisciplinary Research Projects, The University of Tokyo)
Hitomi Kumagai (Associate Professor, Department of Chemistry and Life Science, Nihon University)
Makoto Akao (Assistant Professor, Department of Chemistry and Life Science, Nihon University)
[The National Institute of Health and Nutrition, Japan]
Nobuo Nishi (Director, Center for Collaboration and Partnership, National Institute of Health and Nutrition)
Nobuyo Tsuboyama-Kasaoka (Project leader, Project for International Research and Development, National Institute of Health and Nutrition)
3) Sponsors
Ajinomoto Co., Ltd., Kikkoman Co., Ltd., House Foods Corporation, Kagome Co., Ltd., Kao Corporation, Fuji Oil Co., Ltd., Meiji Dairies Corporation, Morinaga Milk Industry Co., Ltd., Nisshin Seifun Group Inc., Nestlé Japan, Ltd., Yakult Honsha Co., Ltd., Megmilk Snowbrand Co., Ltd.

Table 2. Selected topics for group work and field trips.

Topics	Objectives	Field trip	Group work
Shokuiku at the national level: A focus on school meal programs and related policies	—To understand nutrition education methodology (in particular, the school lunch system) in each country —To understand the historical background of the Japanese school lunch system, as well as nutrition education methodology (including hygiene management) in Japan —To learn national legislation related to *Shokuiku*, and to understand the roles of registered dietitians/dietitians as key players in *Shokuiku*.	Observation of school the lunch system and facilities at an elementary school, and its tasting in a classroom	1) A brief lecture on overviews of the school lunch system provided before the field trip 2) Overseas participants and Japanese participants work in pairs, and discuss the possible activity in this field in each country
Nutrition and Community Empowerment	—To propose a framework for the assessment, monitoring and evaluation of community-based activity towards nutritional improvement —To learn the process to formulate a framework by utilizing past experience (where assessment was incorporated) of each participant —To discuss important views at each level (community, country, regional, global) using the framework formulated	Observation of a community assessment scheme at municipal health centers	1) Exchange information on an internationally used approach for assessment at the community level 2) Exchange opinions on important views for assessment at the community level 3) Incorporate findings of the field trip in 1 and 2 4) Formulate a framework with important views for assessment 5) Prepare a proposal to conduct assessment in the participant's country using the framework in 4
Functional Food	—To understand overviews of functional food and discuss "What is required in a functional food?" (e.g. type of function, safety of functional food) —To understand overviews of Food for Specified Health Uses (FOSHU) and the food labeling system in Japan, and discuss its significance, current problem and challenges —To understand the structure of functional components and their mechanisms	Introduction and tour of a factory at a food technology development center	1) Introduce the current situation of food research and related policies in each participant's country (including the FOSHU system in Japan) 2) Discuss the safety of functional food 3) Outline the type and structure of functional components and their mechanisms

Table 3. Timetable of the program.

September 7 (Tue)	September 8 (Wed)	September 9 (Thurs)
	8:00–8:30 LECTURE (1) Functional food—Concepts and present status in Japan (Prof. Makoto Shimizu, The University of Tokyo)	8:00–8:30 LECTURE (2) Assessing Food and Nutrition Needs in the Community (Kumi Eto, Kagawa Nutrition University)
9:30–10:30 Opening address (Prof. Makoto Shimizu, Representative, IUNS Committee of Science Council of Japan) Lecture/Orientation: Towards more integrated researches on nutrition in Asian regions (Prof. Nobuo Yoshiike, Aomori University of Health and Welfare)	8:30–10:30 GROUP WORK (3)	8:30–15:30 GROUP WORK (5)
10:30–11:30 GROUP WORK (1)	10:45–13:15 FIELD TRIP (2): Ochanomizu University Elementary School	
12:30–13:00 Orientation for field trip		
13:00–18:00 FIELD TRIP (1): i) Nutrition and Community Empowerment: Second Harvest Japan ii) Functional Food: Factories and the Food Development & Technology Center of Ajinomoto Co., Inc.	13:15–13:45 Introduction of NIHN	
	14:00–17:30 The 4th Asian Network Symposium on Nutrition (NIHN)*	
		16:00–18:00 GROUP PRESENTATION
18:00– GROUP WORK (2)	18:00– GROUP WORK (4)	18:00–19:00 Farewell party and closing Closing address (Prof. Chigusa Date, Director, The Japanese Society of Nutrition and Dietetics)

*NIHN: National Institute of Health and Nutrition.

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RESEARCH ARTICLE Open Access

Vitamin A deficiency during pregnancy of HIV infected and non-infected women in tropical settings of Northwest Ethiopia

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Abstract

Background: Vitamin A deficiency (VAD) is known to be a major public health problem among women of reproductive age in South East Asia and Africa. In Ethiopia, there are no studies conducted on serum vitamin A status of HIV-infected pregnant women. Therefore, the present study was aimed at determining the level of serum vitamin A and VAD among pregnant women with and without HIV infection in tropical settings of Northwest Ethiopia.

Methods: In this cross-sectional study, blood samples were collected from 423 pregnant women and from 55 healthy volunteers who visited the University of Gondar Hospital. Serum concentration of vitamin A was measured by high performance liquid chromatography.

Results: After controlling for total serum protein, albumin and demographic variables, the mean ± SD serum vitamin A in HIV seropositive pregnant women (0.96 ± 0.42 μmol/L) was significantly lower than that in pregnant women without HIV infection (1.10 ± 0.45 μmol/L, $P < 0.05$). Likewise, the level of serum vitamin A in HIV seropositive non-pregnant women (0.74 ± 0.39) was significantly lower than that in HIV negative non-pregnant women (1.18 ± 0.59 μmol/L, $P < 0.004$). VAD (serum retinol < 0.7 μmol/L) was observed in 18.4% and 17.7% of HIV infected and uninfected pregnant women, respectively. Forty six percent of non-pregnant women with HIV infection had VAD while only 28% controls were deficient for vitamin A ($P = 0.002$).

Conclusion: The present study shows that VAD is a major public health problem among pregnant women in the tropical settings of Northwest Ethiopia. Considering the possible implications of VAD during pregnancy, we recommend multivitamin (which has a lower level of vitamin A) supplementation in the care and management of pregnant women with or without HIV infection.

Keywords: Vitamin A deficiencies, pregnancy, HIV infection, Ethiopia

Background

Vitamin A deficiency (VAD) is known to be a significant public health problem around the world and it is particularly serious among women of reproductive age in South-East Asia and Africa [1-4]. It has now become evident that VAD in women has negative consequences on their health status as well as on their infants [3,4]. The link between VAD morbidity and mortality from infectious diseases [5] and non-infectious diseases [6-8] has been known for several years.

VAD in pregnant women is associated with night blindness, severe anaemia, wasting, malnutrition, and reproductive and infectious morbidity [9], and increased risk of mortality 1-2 years following delivery [4]. VAD reduces lymphocyte response [10], and also leads to reduced levels of secretory IgA in mucous membranes and therefore weakens the local barriers to infection

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[7,11,12]. As a result, vitamin A deficient women were found to be more susceptible to illnesses of both infectious such as frequent infection of mucous surface of hallow viscera [4] and non-infectious (eclampsia, pre-eclampsia, premature rupture of membrane) diseases [6,13,14].

In the era of HIV/AIDS, vitamin A was also postulated to reduce mother to child transmission (MTCT) of HIV by affecting several maternal, fetal, and/or child risk factors for transmission, including the clinical, immunological, or viral stage of HIV disease among pregnant women. Vitamin A has an effect on the integrity of the epithelial lining of the placenta, maternal lower genital tract, or breast. Its deficiency leads to the occurrence of prematurity and low birth weight, and the status of the systemic and digestive mucosal immune systems of the fetus and the child [15,16]. Observational studies in sub-Saharan Africa have shown that, low serum vitamin A levels in HIV-infected women to be associated with significantly increased rates of MTCT of HIV [17,18] and infant mortality [17,19]. On the contrary, randomized trials of vitamin A supplementation have found that, vitamin A supplementation increases the risk of MTCT [20,21] and can increase mortality in some children born to HIV positive mothers. Vitamin A supplementation in HIV-infected children, on the other hand, has been associated with protective effects against mortality and morbidity, similar to that seen in HIV-negative children [22].

In Ethiopia, studies on VAD in the general population and among pregnant women are scant, despite decades of documentation of VAD as a major public health problem affecting up to 40% of pregnant women [23-25]. Recently, VAD has also been reported as a severe public health problem among tuberculosis [26] and diarrheic [27] patients infected with HIV. There are no studies examining serum vitamin A status of HIV-infected pregnant women in northwest Ethiopia. Therefore, the present study aimed to determine the level of serum vitamin A among pregnant women with and without HIV infection in tropical settings of Northwest Ethiopia.

Methods

Subjects and settings

Pregnant women who visited the antenatal clinic of the University of Gondar Hospital between March and June 2005 in their first trimester for routine antenatal care follow-ups were approached for recruitment. The Hospital is a tertiary level teaching and service rendering hospital that provides health service for over 5 million inhabitants in Northwest Ethiopia. Healthy non pregnant women, who were living in the same geographic locale as pregnant participants, were recruited from voluntary blood donors and served as control subjects. Informed consent was obtained from the study participants and approved by the Research Ethics Committee of the University of Gondar. None of the subjects had cirrhosis and none of them received vitamin A supplementation.

Anthropometric data

Body weight and height was measured to the nearest 0.1 kg on an electronic digital scale and to the nearest 0.1 cm, respectively. Body mass index (BMI) was calculated and used to determine the nutritional status of the study subjects [28], though it is not a surrogate for nutritional assessment during pregnancy.

Blood collection and HIV screening

About 5 ml of venous blood was collected from each subject as per the routine antenatal care follow up of the pregnant women in the morning but fasting status was not ascertained. After clot was retracted, the blood samples were centrifuged and sera were separated from the cells following standard procedures and stored at -40°C until tested. The sera were tested for presence of HIV-1 antibodies using rapid HIV-1 diagnostic test kits (Abbott, Belgium) with a sensitivity of 99.9% and specificity of 98% following the manufacturers' instructions. The results were interpreted following the current national algorithm for screening of HIV-1 infection [29]. CD4 cells count was not measured and none of the study subjects was on antiretroviral therapy because of lack of access in the setting during the study period. Pre and post HIV test counselling was provided to patients and controls as per the routine program.

Biochemical analysis

Serum level of retinol (vitamin A), albumin, total protein and total cholesterol were determined following standard procedures and briefed as follows:

Serum vitamin A: Serum retinol was determined using high performance liquid chromatography (HPLC) according to the method of Arroyave *et al* [30] utilizing a Shiseido HPLC system (Shiseido Co. Ltd., Tokyo, Japan) which consisted of a separation by a reverse phase column (Capcell Pak C18 MG S-5, 3 × 250 mm, 5 μm, Shiseido, Japan) and ultraviolet detection using methanol as a mobile phase system. The flow rate was adjusted at 500 μl/min with column temperature set at 40°C. Serum was deproteinised with an alcoholic solution of retinyl acetate and extracted into hexane. The organic layer was separated, evaporated to dryness under a steam of nitrogen, reconstituted in methanol and injected into the HPLC system with a detection wave length set at 325 nm. All extraction procedures were carried out under reduced light in order to prevent oxidation of the compounds. Pooled human sera were